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Growth Performance and Proximate and Fatty Acid Compositions of Channel Catfish, *Ictalurus punctatus*, Fed for Different Duration with a Commercial Diet Supplemented with Various Levels of Menhaden Fish Oil

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Abstract

A 15-wk study was conducted to evaluate the effect of supplemental menhaden fish oil levels and feeding duration on growth performance and tissue proximate and fatty acid (FA) compositions of juvenile channel catfish, *Ictalurus punctatus*. Dietary fish oil levels had no effect on final weight gain, feed efficiency, and survival of channel catfish. Tissue lipid contents were directly correlated to dietary lipid levels, while moisture contents were inversely related to dietary lipid levels. Fillet moisture contents progressively decreased, whereas fillet lipid increased with increasing feeding duration. Significant increase in saturated and total n-3 FAs and decrease in monoenoic and total n-6 FA in whole body and fillet were observed at each incremental level of dietary fish oil. Percentages of n-3 and n-3 highly unsaturated fatty acids in fillet of fish fed the control and 3% fish oil diets decreased with increasing feeding periods, whereas those of fish fed 6 or 9% added fish oil diets remained stable or increased. Ratios of n-3/n-6 were statistically comparable throughout the 15-wk feeding. When expressed in terms of mg/g of fillet, the highest concentration of n-3 was obtained in fillets of fish fed the 9% added fish oil diet for 15 wk.

There is evidence that high levels of omega-3 highly unsaturated fatty acids (n-3 HUFA), which consist mainly of eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), play vital roles in human nutrition, disease prevention, and health promotion (Sidhu 2003). They provide a protective effect in minimizing the development of several chronic degenerative diseases and have a therapeutic effect in certain cases (Magaro et al. 1988; Okuyama et al. 1997; Belch and Muir 1998; Horrocks and Yeo 1999). Humans cannot synthesize *de novo* n-3 HUFA, and these must be obtained through the diet. Marine organisms are the primary sources of these fatty acids (FAs) available to humans. As the general public becomes aware of the health benefits of consuming seafood high in n-3 HUFA, the content of these FAs in aquaculture products could become a major factor in determining consumer acceptance in the future.

Production of channel catfish, *Ictalurus punctatus*, has become the largest aquaculture enterprise in the USA. To ensure continued growth of this industry, quality of channel catfish products has to be maintained and improved, particularly with regard to the quality of lipids. Fish and shellfish ingest and accumulate n-3 FAs through the food chain from algae and phytoplankton, the primary producers of n-3 FAs. Most vegetable oils and fats from domestic animals contain high levels of n-6 FAs and low concentrations of n-3 HUFA. Because of differences in essential FA requirements, cultured freshwater fish, including channel catfish, are commonly fed grain–soybean meal feeds high in n-6 FAs, whereas marine fish are fed diets rich in n-3 HUFA (NRC 1993). However, it has been shown that FA composition of fat in fish tissues is influenced by dietary FA composition (Stickney and Andrews 1971; Gatlin and Stickney 1982; Janncke et al. 1988; Chen et al. 1995; Grigorakis et al. 2002). Channel catfish fed diets supplemented with fish oil had significantly increased concentrations of n-3 HUFA (Abdel-Aty Mohamed 1989; Li et al. 1994; Fracalossi and Lovell 1995; Manning and Li 2002), and the tissue concentrations of n-3 HUFA are

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affected by dietary levels of fish oil (Abdel-Aty Mohamed 1989; Li et al. 1994). However, there is no published information on the effect of feeding duration and dietary fish oil levels on the accumulation of these FAs in fish tissues. This information is essential to optimize the use of marine fish oils, which are a limited resource. Therefore, this study was conducted to evaluate the effect of feeding duration of diets containing various levels of fish oil on n-3 HUFA content in channel catfish fillets. Growth, feed utilization, and whole-body proximate and FA compositions of fish receiving various dietary levels of fish oil for 15 wk were also determined.

Materials and Methods

Experimental Fish and Rearing Facilities

National Warmwater Aquaculture Center 103 strain channel catfish, *I. punctatus*, fingerlings from a single spawn that have been reared at our laboratory on a commercial trout diet from yolk sac fry to juveniles were acclimated to a commercial floating catfish fingerling feed for 2 wk prior to stocking. At the end of the acclimation period, fish (average weight of 14.56 ± 0.23 g) were randomly stocked into sixteen 110-L aquaria at a density of 50 fish per aquarium. Aquaria were supplied with flow-through dechlorinated, heated city water at an initial rate of about 0.7–0.8 L/min, and the rate was increased gradually to about 1.5 L/min prior to the end of the study. Water was continuously aerated using air stones. Water temperature and dissolved oxygen in three randomly chosen aquaria were measured once every other day in the morning using an YSI model 58 Oxygen Meter (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA). During the trial, water temperature averaged 26 ± 0.4 C and dissolved oxygen averaged 4.9 ± 0.04 mg/L. Photoperiod was maintained at a 12:12 h light : dark schedule.

Feeding and Sampling

A commercial floating feed for fingerling catfish containing 35.3% crude protein and 5.6% lipid (Alabama Farmers Cooperative, Inc., Decatur, AL, USA) was sprayed with menhaden fish oil (ARBP refined menhaden oil, Omega protein, Inc., Reedville, VA, USA) at levels of 0, 3, 6, and 9% of diets and thoroughly mixed in a Hobart mixer (Hobart Food Equipment, Troy, OH, USA). Diets were stored in plastic bags at −20 C until used. The proximate and FA compositions of the experimental diets are given in Tables 1 and 2, respectively.

Fish in four randomly assigned aquaria were fed one of the four experimental diets twice daily (between 0730–0830 and 1500–1600 h) to apparent satiation for 15 wk. The amount of diet consumed was recorded daily by calculating the differences in weight of diets prior to the first and after the last feeding. Once a week, aquaria were scrubbed and accumulated wastes siphoned. On cleaning days, fish were fed only in the afternoon. Fish in each aquarium were group weighted and counted at 3-wk intervals. On the sampling days, three fish per tank were removed, weighed, and filleted. Fillets were stored at −80 C until analyzed for proximate and FA compositions. Feed was not offered on sampling days. Fifty fish at the beginning of the trial and four fish from each aquarium at the end of the trial were randomly sampled, pooled, and stored at −80 C for determination of whole-body proximate and FA compositions.

Body Composition and FA Analysis

Each sample was analyzed in duplicate for proximate composition following standard methods (AOAC 1990). Moisture content was

TABLE 1. *Proximate composition (% air dry weight) of experimental diets.*[1]

Proximate constituents	Experimental diets[2]			
	Control-C	C + 3%	C + 6%	C + 9%
Dry matter	93.39	93.73	94.25	94.72
Protein	35.31	34.80	33.89	33.20
Lipid	5.61	8.34	10.59	13.25
Ash	6.92	6.69	6.56	6.27

[1] Values reported are means of two determinations per diet.

[2] C = control diet; C + 3% = control + 3% fish oil; C + 6% = control + 6% fish oil; C + 9% = control + 9% fish oil.

TABLE 2. *Fatty acid composition (% by weight of total fatty acids) of menhaden fish oil and experimental diets.*[1]

	Menhaden fish oil	Experimental diets			
		Control-C	C + 3%	C + 6%	C + 9%
14:0	7.8	1.90	3.35	4.90	5.75
15:0	—	0.20	0.15	0.20	0.40
16:0	22.3	19.95	21.65	22.20	20.75
18:0	3.9	5.65	4.95	4.85	4.50
Total saturates	34.00	27.70	30.10	32.15	31.40
16:1 n-7	13.3	3.25	5.65	7.90	8.40
18:1 n-7	3.8	1.80	2.25	2.75	2.75
18:1 n-9	9.3	24.65	18.80	17.40	16.65
20:1 n-9	1.9	0.50	0.35	0.70	0.95
Total monoenes	28.30	30.20	27.05	28.75	28.75
18:2 n-6	1.3	33.80	27.75	21.80	16.15
20:2 n-6	0.3	0.10	0.10	0.15	0.20
18:3 n-6	0.5	0.05	0.05	0.20	0.20
20:3 n-6	0.2	0.10	0.05	0.10	0.10
20:4 n-6	0.8	0.30	0.20	0.45	0.50
22:5 n-6	0.9	0.10	0.15	0.20	0.05
Total n-6	4.00	34.45	28.30	22.90	17.20
18:3 n-3	0.3	2.65	2.55	2.35	2.00
20:3 n-3	0.2	0.00	0.05	0.10	0.05
18:4 n-3	1.6	0.30	1.10	1.75	2.00
20:4 n-3	2.1	0.20	0.70	0.95	1.10
20:5 n-3	12.4	2.20	4.40	3.15	7.45
22:5 n-3	3.2	0.55	0.60	0.65	1.60
22:6 n-3	14.1	1.65	5.00	7.30	8.40
Total n-3	33.90	7.55	14.40	16.25	22.60
n-3 HUFA[2]	32.00	4.60	10.75	12.15	18.60
n-3/n-6	8.48	0.22	0.51	0.71	1.31

HUFA = highly unsaturated fatty acids.
[1] Values reported are means of two determinations per diet.
[2] n-3 fatty acids with 20 carbons or more.

determined by drying fish samples in an oven at 90 C until constant weight was reached. Samples used for dry matter were digested with nitric acid and incinerated in a muffle furnace at 600 C overnight for measurement of ash contents. Protein was measured by combustion method using an FP-2000 Nitrogen Analyzer (Leco Corp., St. Joseph, MI, USA). Lipid content of the experimental diets and fish tissues (whole body and fillets) was determined following the method of Folch et al. (1957).

The resulting lipids from feeds or fillets were analyzed for their FA composition by gas chromatography. Lipid fractions were dissolved in 2 mL hexane, 0.2 mL benzene, and 2 mL boron trifluoride–methanol solution (Sigma Chemical Co., St. Louis, MO, USA) and esterified by heating in a 95–100 C water bath for 30 min in vials with caps tightened. The vials were allowed to cool to room temperature, and 2 mL hexane and 4 mL of water were added. The vials were vortexed for 1 min and centrifuged at 3000 *g* for 10 min. The hexane layer was pipetted off into a new vial and evaporated under N_2 for 20 min. FA methyl esters were analyzed with a Perkin-Elmer Clarus-500 gas chromatograph with a mass spectrometry detector (Perkin-Elmer, Shelton, CT, USA). The samples in 0.5 μL of hexane with butylated hydroxytoluene were injected onto a 30 m × 0.25 mm × 0.25 μm film thickness capillary column (Omegawax, Supelco, Bellefonte, PA, USA). Temperature program conditions were as follows: injector, 260 C; ramp 1, 90–140 C at 5 C/min; ramp 2, 140–240 C at 2 C/min; and held at 240 C for 5 min. Peaks were detected and identified in total ion mode using Turbomass software (Perkin-Elmer) based on retention times and mass spectra compared with analytical standards (Supelco 37, Supelco). Relative concentrations were calculated and expressed as mass percentages of the identified FAs.

Statistical Analysis

Data on growth performance, whole-body proximate composition, and whole-body FA composition were analyzed by one-way ANOVA using the general linear model. If there was a significant F-test, subsequent comparisons of treatment means were determined using the Duncan's multiple range test. Data on proximate composition and the sum of major FAs (saturates, monoenes, n-6, n-3, n-3/n-6, and n-3 HUFA) of fillets and levels of n-3 per unit of fillets sampled at Weeks 3, 6, 9, 12, and 15 were subjected to two-way ANOVA to test effects of dietary fish oil levels and feeding duration. Differences were considered significant at the 0.05 probability level. All analyses were performed using the SAS program (Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, USA, 1999–2001).

Results

Growth, Feed Utilization, and Proximate Composition of Whole Body and Fillets

Final weight gain, dry matter feed intake, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival of juvenile channel catfish fed a commercial diet supplemented with various levels of menhaden fish oil for 15 wk are presented in Table 3. No significant differences were observed among the values of these parameters for fish receiving various dietary treatments.

Whole-body moisture significantly decreased, whereas lipid increased at each incremental level of additional fish oil (Table 4). Whole-body protein of fish fed the diet supplemented with 6% fish oil was significantly lower than that of fish fed the control diet (0% supplemental fish oil) but did not differ from that of the group receiving the diet with 3% added fish oil. Fish fed the diet supplemented with 9% fish oil had significantly lowest body protein. No significant differences were observed in whole-body ash content of fish receiving various dietary treatments.

Proximate composition of fillets in relation to dietary levels of supplemental fish oil and feeding durations is given in Table 5. Fillets of fish fed the 9% supplemental fish oil diet had significantly lower moisture and higher lipid than those fed lower dietary fish oil levels. The values of these parameters for fish receiving the 3 and 6% supplemental fish oil diets did not significantly differ, but these were significantly different from those fed the control diet (0% fish oil). Fillet protein contents of fish fed the 6 and 9% supplemental fish oil diet were statistically similar but were significantly lower than those fed

TABLE 3. *Mean final body weight gain (WG), dry matter feed intake (FI), feed efficiency ratio (FER), protein efficiency ratio (PER), and survival of channel catfish fed commercial diets supplemented with various levels of menhaden fish oil for 15 wk.*[1]

Level of fish oil added (%)	WG (g)	FI (g)	FER[2]	PER[3]	Survival (%)
0	103.52	123.70	0.82	2.02	100.0
3	101.01	122.67	0.82	2.00	95.5
6	95.45	118.36	0.81	1.99	98.0
9	111.27	125.95	0.88	2.37	97.0
Pooled SEM	4.86	3.07	0.03	1.10	0.41

SEM = standard error of the mean.

[1] Values are means of four replicates per treatment. No significant differences were detected among treatment means of various parameters ($P < 0.05$).

[2] FER = weight gain (g)/dry feed fed (g).

[3] PER = wet weight gain (g)/crude protein fed (g).

TABLE 4. *Whole-body proximate composition of channel catfish fed commercial diets supplemented with various levels of menhaden fish oil for 15 wk.*[1]

Levels of fish oil added (%)	Moisture (%)	Percent wet weight basis		
		Protein	Lipid	Ash
0	71.41[a]	15.51[a]	9.58[d]	2.91
3	70.21[b]	15.31[ab]	11.36[c]	2.82
6	69.13[c]	14.83[b]	12.84[b]	2.75
9	67.99[d]	14.24[c]	13.84[a]	2.62
Pooled SEM	0.33	1.19	0.30	0.16

SEM = standard error of the mean.

[1] Values are means of two determinations of pooled samples of four fish per tank and four tanks per treatment. Means in the same column with different superscripts are significantly different at $P < 0.05$.

TABLE 5. *Proximate composition of catfish fillet after 3, 6, 9, 12, and 15 wk of feeding with a commercial diet supplemented with various levels of menhaden fish oil.*[1]

	Moisture (%)	Percent wet weight basis		
		Protein	Lipid	Ash
Fish oil effect (%)				
0	78.95[a]	17.16[a]	3.21[c]	1.16
3	78.46[b]	17.05[ab]	3.85[b]	1.14
6	78.30[b]	16.86[bc]	4.12[b]	1.14
9	77.90[c]	16.71[c]	4.58[a]	1.21
P level	<0.0001	<0.0001	<0.0001	0.0991
Feeding period effect (wk)				
3	80.58[a]	16.09[b]	2.53[d]	1.09[b]
6	78.50[b]	17.25[a]	3.44[c]	1.12[b]
9	77.90[c]	17.17[a]	4.14[b]	1.15[a]
12	77.64[cd]	17.16[a]	4.48[b]	1.16[a]
15	77.29[d]	17.06[a]	5.11[a]	1.18[a]
P level	<0.0001	0.0023	<0.0001	0.0001
Fish oil × feeding period (*P* level)	0.5827	0.9928	0.9908	0.376
Pooled SEM	0.306	0.194	0.360	0.022

SEM = standard error of the mean.

[1] Values are means of two determinations of pooled samples of fillet from three fish per tank and four tanks per treatment. Means in the same column with different superscripts are significantly different at $P < 0.05$.

the control diet. Fillet protein of fish fed the 3% added fish oil diet did not differ from that of fish fed the control and 6% fish oil diets but was significantly higher than that fed the 9% fish oil diet. Dietary fish oil levels had no effect on fillet ash content. Feeding durations also significantly affected fillet proximate composition (Table 5). Increasing feeding duration resulted in decreasing moisture and increasing protein, lipid, and ash. The differences between the values at various time periods, however, were not always significant. There was no significant interaction between dietary fish oil levels and feeding duration on fillet proximate composition.

FA Composition of Whole-Body and Fillet Lipids

FA composition of whole-body lipid, expressed as percent of total FA of channel catfish at the beginning of the experiment (initial fish) and after 15 wk of feeding diets supplemented with various levels of fish oil, is given in Table 6. Fish fed the control diet had similar FA profiles to those of the initial fish. Increasing supplemental levels of dietary fish oil significantly increased whole-body total saturate, n-3, n-3 HUFA, and n-3/n-6 ratio but significantly decreased the total monoenoic and n-6 series FA. However, the value of the total saturated FA in fish fed the 3% supplemental fish oil diet did not significantly differ from the group fed the 6% added fish oil diet.

TABLE 6. *Whole-body fatty acid composition (% by weight of total fatty acids) of catfish before and after feeding for 15 wk with a commercial diet supplemented with various levels of menhaden fish oil.*[1]

	Experimental diets				
	Initial	Control-C	C + 3%	C + 6%	C + 9%
14:0	1.85	1.83	3.05	3.86	5.01
15:0	0.16	0.16	0.23	0.30	0.36
16:0	21.23	20.95	21.24	20.97	21.72
18:0	4.09	4.06	4.34	4.55	4.49
Total saturates	27.33[c]	27.00[c]	28.86[b]	29.68[b]	31.58[a]
16:1 n-7	4.14	4.11	5.06	5.91	6.74
18:1 n-7	1.84	1.90	2.10	2.31	2.50
18:1 n-9	43.05	42.91	38.17	33.67	30.89
20:1 n-9	1.17	1.20	1.09	1.09	1.09
Total monoenes	50.19[a]	50.12[a]	46.42[b]	42.97[c]	41.22[d]
18:2 n-6	15.48	15.47	13.86	12.40	10.87
20:2 n-6	0.22	0.23	0.16	0.13	0.15
18:3 n-6	0.99	1.02	0.63	0.58	0.46
20:3 n-6	0.86	0.85	0.49	0.36	0.34
20:4 n-6	0.27	0.46	0.42	0.48	0.45
22:5 n-6	0.01	0.01	0.01	0.01	0.02
Total n-6	17.83[a]	18.04[a]	15.57[b]	13.96[c]	12.28[d]
18:3 n-3	0.97	0.99	1.11	1.18	1.21
20:3 n-3	0.50	0.35	0.53	0.86	0.95
18:4 n-3	0.04	0.26	0.39	0.56	0.26
20:4 n-3	0.24	0.25	0.67	0.99	1.14
20:5 n-3	0.58	0.68	1.74	2.95	3.56
22:5 n-3	0.45	0.43	0.83	1.15	1.39
22:6 n-3	1.71	1.73	3.67	5.44	6.11
Total n-3	4.49[d]	4.69[d]	8.93[c]	13.14[b]	14.63[a]
n-3 HUFA	2.98[d]	3.08[d]	6.9 [c]	10.53[b]	12.20[a]
n-3/n-6	0.25[d]	0.26[d]	0.57[c]	0.94[b]	1.19[a]

HUFA = highly unsaturated fatty acids.

[1] Values are means of eight determinations per treatment (two determinations of pooled samples of four fish per tank and four tanks per treatment). Means in the same row with different superscripts are significantly different at $P < 0.05$.

Fillet FA composition of fish after feeding various dietary levels of supplemental fish oil for 3, 6, 9, 12, and 15 wk is presented in Tables 7, 8, 9, 10, and 11, respectively. After 3 wk of feeding various dietary fish oil levels, there were no significant changes in the fillet

TABLE 7. *Fatty acid composition (% by weight of total fatty acids) of fillet of catfish after feeding 3 wk with a commercial diet supplemented with various levels of menhaden fish oil.*[1]

	Experimental diets				
	Initial	Control-C	C + 3%	C + 6%	C + 9%
14:0	1.38	2.47	2.59	3.04	3.66
15:0	0.23	0.19	0.22	0.27	0.26
16:0	23.41	25.51	25.96	25.50	26.12
17:0	0.28	0.20	0.22	0.26	0.26
18:0	9.15	6.75	7.46	8.12	7.25
Total saturates	34.45	35.12[a]	36.45[a]	37.19[a]	37.55[a]
16:1 n-7	2.35	4.54	4.38	4.63	5.30
18:1 n-7	2.32	2.22	2.23	2.28	2.37
18:1 n-9	24.77	33.60	30.75	27.65	27.55
20:1 n-9	1.56	0.91	0.85	0.85	0.84
Total monoenes	31.00	41.27[a]	38.21[a]	35.41[a]	36.06[a]
18:2 n-6	14.84	11.95	11.40	10.44	10.20
20:2 n-6	1.57	1.15	1.05	0.93	1.23
18:3 n-6	0.51	0.01	0.01	0.03	0.08
20:3 n-6	2.37	0.71	0.70	0.59	0.66
20:4 n-6	3.06	1.00	1.08	1.19	1.11
22:5 n-6	1.27	0.26	0.31	0.50	0.43
Total n-6	23.62	15.08[a]	14.55[a]	13.68[a]	13.71[a]
18:3 n-3	0.68	0.81	0.80	0.86	0.87
20:3 n-3	0.54	0.19	0.25	0.31	0.32
18:4 n-3	0.17	0.00	0.00	0.00	0.00
20:4 n-3	1.59	0.45	0.53	0.74	0.74
20:5 n-3	0.01	1.93	2.33	3.23	3.20
22:5 n-3	0.88	0.73	0.92	1.12	1.08
22:6 n-3	7.04	4.41	5.98	7.44	6.47
Total n-3	10.91	8.52[a]	10.8[a]	13.70[a]	12.68[a]
n-3 HUFA	10.06	7.71[a]	10.00[a]	12.84[a]	11.81[a]
n-3/n-6	0.46	0.56[a]	0.74[a]	1.00[a]	0.92[a]

HUFA = highly unsaturated fatty acids.

[1] Values are means of one determination per pooled sample of four fish per tank and four tanks per treatment. Means in the same row with different superscripts are significantly different at $P < 0.05$.

TABLE 8. *Fatty acid composition (% by weight of total fatty acids) of fillet of catfish after feeding 6 wk with a commercial diet supplemented with various levels of menhaden fish oil.*[1]

	Experimental diets			
	Control-C	C + 3%	C + 6%	C + 9%
14:0	1.83	2.89	3.96	4.71
15:0	0.20	0.33	0.40	0.43
16:0	24.71	25.23	24.87	24.74
17:0	0.17	0.20	0.23	0.28
18:0	5.90	6.71	6.13	6.13
Total saturates	32.81[b]	35.36[a]	35.59[a]	36.29[a]
16:1 n-7	4.10	4.47	5.57	6.15
18:1 n-7	1.92	2.05	2.25	2.42
18:1 n-9	37.17	32.04	30.36	27.70
20:1 n-9	1.00	0.93	0.86	0.86
Total monoenes	44.19[a]	39.49[b]	39.04[b]	37.13[b]
18:2 n-6	13.73	11.85	10.51	9.15
20:2 n-6	1.28	0.95	0.69	0.74
18:3 n-6	0.15	0.13	0.27	0.20
20:3 n-6	1.03	0.46	0.26	0.13
20:4 n-6	0.85	0.75	0.69	0.68
22:5 n-6	0.40	0.37	0.41	0.39
Total n-6	17.44[a]	14.51[b]	12.83[c]	11.29[d]
18:3 n-3	0.88	0.94	1.01	1.02
20:3 n-3	0.29	0.43	0.30	0.37
18:4 n-3	0.13	0.27	0.51	0.68
20:4 n-3	0.29	0.62	0.78	1.01
20:5 n-3	0.79	2.06	2.58	3.69
22:5 n-3	0.52	0.88	1.14	1.32
22:6 n-3	2.66	5.46	6.22	7.19
Total n-3	5.56[d]	10.66[c]	12.54[b]	15.28[a]
n-3 HUFA	4.55[d]	9.45[c]	11.02[b]	13.58[a]
n-3/n-6	0.32[d]	0.73[c]	0.98[b]	1.35[a]

HUFA = highly unsaturated fatty acids.

[1] Values are means of one determination per pooled sample of four fish per tank and four tanks per treatment. Means in the same row with different superscripts are significantly different at $P < 0.05$.

content of total saturates, monoenes, n-6, n-3, n-3 HUFA, and n-3/n-6 ratio. At Week 6, total saturated and monoenoic FA of fish fed diets containing added fish oil significantly decreased and increased, respectively, as compared to those of the control. The values of total n-6 significantly decreased, whereas total n-3, n-3 HUFA, and n-3/n-6 significantly increased at each incremental level of fish oil. At the end of Weeks 9, 12, and 15, the values of all these major FAs followed the same trend as those observed at Week 6, except that the differences among the values of some FAs in fillets of fish receiving supplemental fish oil diets were not always significant.

When pooled by dietary fish oil levels (Fig. 1), the values of major classes of FA in fillets of fish fed different dietary fish oil levels for various durations appeared to follow a similar trend as those of the whole-body lipid at Week 15. Total saturate, n-3, n-3 HUFA, and n-3/n-6 ratio significantly increased, whereas monoenoic and n-6 FA decreased with increasing dietary fish oil levels. Feeding duration had no effect on total n-3 FA and the n-3/n-6 ratio but significantly affected saturated, monoenoic, n-6

TABLE 9. *Fatty acid composition (% by weight of total fatty acids) of fillet of catfish after feeding 9 wk with a commercial diet supplemented with various levels of menhaden fish oil.*[1]

	Experimental diets			
	Control-C	C + 3%	C + 6%	C + 9%
14:0	1.79	2.93	3.73	4.34
15:0	0.42	0.43	0.51	0.53
16:0	23.64	23.96	24.03	23.87
17:0	0.13	0.19	0.23	0.27
18:0	5.63	5.86	6.25	6.21
Total saturates	31.61[c]	33.37[b]	34.75[a]	35.22[a]
16:1 n-7	3.59	4.50	5.13	5.74
18:1 n-7	1.86	2.05	2.22	2.43
18:1 n-9	36.60	33.68	30.25	27.36
20:1 n-9	1.14	0.98	0.94	1.02
Total monoenes	43.19[a]	41.21[a]	38.54[b]	36.55[b]
18:2 n-6	13.74	12.01	10.28	9.31
20:2 n-6	1.39	0.86	0.73	0.70
18:3 n-6	2.96	2.25	2.13	2.19
20:3 n-6	0.82	0.26	0.20	0.18
20:4 n-6	0.86	0.56	0.65	0.77
22:5 n-6	0.39	0.28	0.23	0.29
Total n-6	20.16[a]	16.22[b]	14.22[c]	13.44[d]
18:3 n-3	0.93	1.02	1.03	1.02
20:3 n-3	0.29	0.34	0.48	0.44
18:4 n-3	0.07	0.32	0.54	0.66
20:4 n-3	0.23	0.59	0.78	0.96
20:5 n-3	0.17	1.30	2.33	2.99
22:5 n-3	0.41	0.75	1.02	1.22
22:6 n-3	2.95	4.89	6.31	7.52
Total n-3	5.05[c]	9.21[b]	12.49[a]	14.81[a]
n-3 HUFA	4.05[c]	7.87[b]	10.92[a]	13.13[a]
n-3/n-6	0.25[c]	0.57[b]	0.88[a]	1.10[a]

HUFA = highly unsaturated fatty acids.

[1] Values are means of one determination per pooled sample of four fish per tank and four tanks per treatment. Means in the same row with different superscripts are significantly different at $P < 0.05$.

TABLE 10. *Fatty acid composition (% by weight of total fatty acids) of fillet of catfish after feeding 12 wk with a commercial diet supplemented with various levels of menhaden fish oil.*[1]

	Experimental diets			
	Control-C	C + 3%	C + 6%	C + 9%
14:0	1.70	2.94	4.01	4.67
15:0	0.33	0.34	0.48	0.48
16:0	23.56	23.78	24.20	24.41
17:0	0.23	0.08	0.23	0.13
18:0	5.47	5.57	5.77	5.83
Total saturates	31.30[b]	32.71[b]	34.69[a]	35.53[a]
16:1 n-7	3.56	4.62	5.40	6.05
18:1 n-7	1.87	2.07	2.21	2.42
18:1 n-9	39.41	35.57	32.04	28.96
20:1 n-9	1.15	1.05	1.01	1.01
Total monoenes	45.99[a]	43.31[b]	40.66[c]	38.45[d]
18:2 n-6	14.07	12.18	10.68	9.64
20:2 n-6	1.04	0.67	0.46	0.45
18:3 n-6	1.61	0.99	1.50	1.12
20:3 n-6	0.77	0.30	0.11	0.15
20:4 n-6	0.68	0.52	0.47	0.55
22:5 n-6	0.19	0.18	0.17	0.21
Total n-6	18.36[a]	14.83[b]	13.39[c]	12.14[d]
18:3 n-3	0.90	0.94	1.05	1.06
20:3 n-3	0.25	0.29	0.29	0.37
18:4 n-3	0.14	0.33	0.52	0.75
20:4 n-3	0.22	0.59	0.76	0.91
20:5 n-3	0.36	1.62	2.13	3.02
22:5 n-3	0.24	0.75	0.91	1.20
22:6 n-3	2.25	4.62	5.61	6.58
Total n-3	4.35[d]	9.14[c]	11.27[b]	13.89[a]
n-3 HUFA	3.32[d]	7.87[c]	9.69[b]	12.08[a]
n-3/n-6	0.24[d]	0.62[c]	0.84[b]	1.14[a]

HUFA = highly unsaturated fatty acids.

[1] Values are means of one determination per pooled sample of four fish per tank and four tanks per treatment. Means in the same row with different superscripts are significantly different at $P < 0.05$.

and n-3 HUFA (Fig. 2). Saturated FA significantly decreased at Weeks 6 and 9, but no further decrease was observed between Weeks 9 and 15. Monoenoic FA significantly increased with increasing feeding duration. However, no significant differences were observed among the values at Weeks 6 and 9 and Weeks 12 and 15. Total linolenic series (n-6) FA of fillets at Weeks 3, 6, 12, and 15 did not significantly differ, but these were significantly lower than that of the fillets at Week 9. Fillet n-3 HUFA at Weeks 9, 12, and 15 were not significantly different but were significantly lower than that of the fillets at Week 3. The value of these FA at Week 6 was significantly higher than that at Week 12 but did not differ from those at Weeks 9 and 15. There were no significant interactions between dietary fish oil levels and feeding durations among the total concentrations of saturated, monoenoic, total n-3, n-3 HUFA, and the ratio of n-3/n-6 FA. Significant interactions between dietary fish oil levels and feeding duration were observed among fillet n-6 FA.

Total n-3 FA content expressed as mg/g of fillet significantly increased at each incremental level of added fish oil (Fig. 3). Increasing feeding duration also significantly increased total n-3 concentrations in fillets. Significantly higher

TABLE 11. *Fatty acid composition (% by weight of total fatty acids) of fillet of catfish after feeding 15 wk with a commercial diet supplemented with various levels of menhaden fish oil.*[1]

	Experimental diets			
	Control-C	C + 3%	C + 6%	C + 9%
14:0	1.70	3.05	3.87	4.58
15:0	0.37	0.36	0.43	0.50
16:0	23.25	23.99	23.76	23.66
17:0	0.18	0.24	0.29	0.36
18:0	5.18	5.27	5.68	5.50
Total saturates	30.68[c]	32.90[b]	34.03[a]	34.60[a]
16:1 n-7	3.42	4.58	5.21	5.90
18:1 n-7	1.82	2.01	2.27	2.45
18:1 n-9	38.48	35.82	31.35	29.12
20:1 n-9	1.26	1.10	1.12	1.09
Total monoenes	44.98[a]	43.50[a]	39.95[b]	38.56[b]
18:2 n-6	13.93	12.48	11.28	9.50
20:2 n-6	1.39	0.76	0.70	0.68
18:3 n-6	1.11	0.62	0.65	0.84
20:3 n-6	0.71	0.19	0.28	0.17
20:4 n-6	0.82	0.50	0.59	0.65
22:5 n-6	0.41	0.21	0.17	0.28
Total n-6	18.36[a]	14.76[b]	13.67[c]	12.12[d]
18:3 n-3	0.87	1.01	1.06	1.06
20:3 n-3	0.47	0.30	0.33	0.99
18:4 n-3	1.21	0.38	0.50	0.51
20:4 n-3	0.22	0.54	0.81	0.74
20:5 n-3	0.58	1.92	2.62	3.42
22:5 n-3	0.43	0.58	1.06	1.29
22:6 n-3	2.21	4.07	5.96	6.70
Total n-3	5.99[d]	8.80[c]	12.34[b]	14.71[a]
n-3 HUFA	3.91[d]	7.41[c]	10.78[b]	13.14[a]
n-3/n-6	0.33[d]	0.60[c]	0.90[b]	1.21[a]

HUFA = highly unsaturated fatty acids.

[1] Values are means of one determination per pooled sample of four fish per tank and four tanks per treatment. Means in the same row with different superscripts are significantly different at $P < 0.05$.

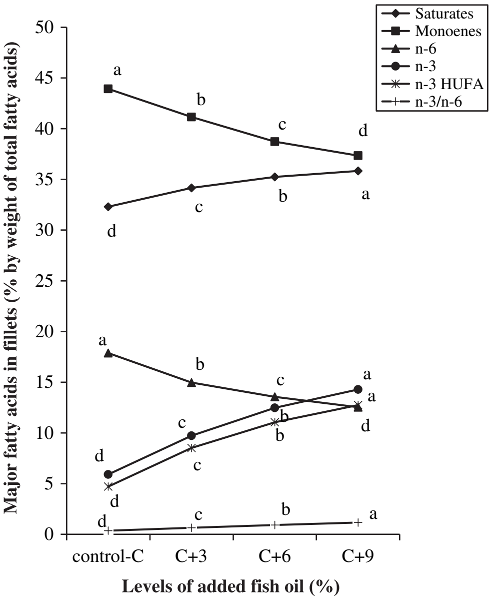

FIGURE 1. *Composition of major fatty acid in fillets of channel catfish fed a commercial diet supplemented with various levels of menhaden fish oil for 15 wk.*

n-3 levels were obtained in fillets of fish at the end of Week 15. Fillet n-3 levels at Weeks 9 and 12 were similar but were significantly higher than those at Weeks 3 and 6. There were no significant differences between n-3 levels of fillets at Weeks 3 and 6. The interaction between dietary fish oil levels and feeding duration, however, was not significant.

Discussion

Results of a number of earlier studies with fish have shown improved growth and feed utilization efficiency with increasing dietary lipid up to certain levels (Williams and Robinson 1988; Santha and Gatlin 1991; Gatlin and Bai 1993; Chou and Shiau 1996; Twibell and Wilson 2003) and beyond which growth depression

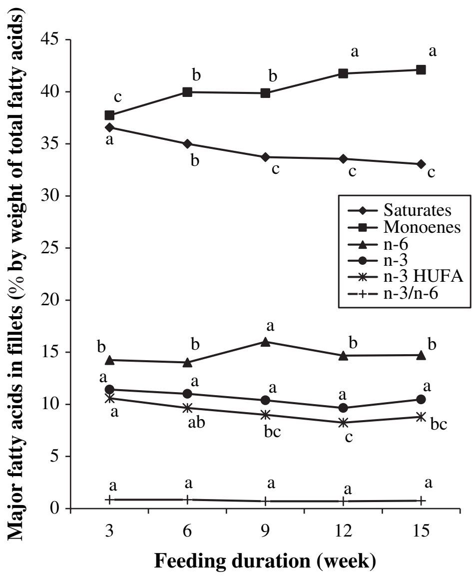

FIGURE 2. *Composition of major fatty acid in fillets of channel catfish after feeding for various periods with a commercial diet supplemented with various level of fish oil.*

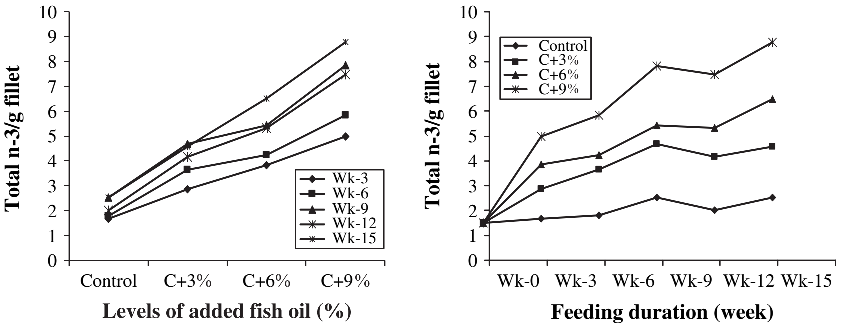

FIGURE 3. *Effects of feeding diets supplemented with various levels of fish oil (a) and of feeding duration (0, 3, 6, 9, 12, and 15 wk) (b) on total n-3 content in fillets (mg/g).*

occurred (Williams and Robinson 1988; Santha and Gatlin 1991; Chou and Shiau 1996). In the present study, however, supplementation of menhaden fish oil at 0, 3, 6, and 9% to a commercial floating catfish diet containing 5.61% lipid had no significant effect on weight gain, FER, and PER. This is consistent with the findings of Lim et al. (2006) who observed no significant differences among weight gain and feed efficiency of channel catfish fed purified diets supplemented with menhaden fish oil at levels ranging from 6 to 14%. Gatlin and Stickney (1982) also reported no significant growth differences over a 20-wk feeding of juvenile channel catfish with semipurified diets containing beef tallow, soybean oil, or fish oil at levels ranging from 6 to 14%. In European sea bass, *Dicentrarchus labrax*, Peres and Oliva-Teles (1999a) obtained no differences in growth rate and feed efficiency of fish fed diets containing lipid (cod liver oil) at levels ranging from 12 to 24%.

Fish like homoeothermic animals adjust feed intake to satisfy their energy requirements (NRC 1993). Mangalik (1986) found that increasing the digestible energy level of a 27% protein diet reduced weight gain in channel catfish because of decreased feed intake. Santha and Gatlin (1991) reported decreased feed intake in catfish fed the diet in which 6% menhaden fish oil was added to the basal diet containing 3% lipid. Lipid concentrations in commercial channel catfish diets are generally limited to 5–6% as higher levels can result in reduced feed consumption (Wilson and Moreau 1996). In our study, however, increasing dietary lipid levels of a 35% protein commercial catfish diet from 5.6 to 13.3% had no effect on feed intake. Similar results have been reported for the same species by Twibell and Wilson (2003) and Lim et al. (2006). In European sea bass, a significant reduction of feed intake occurred only when dietary lipid levels were increased from 24 to 30% but not from 12 to 24% (Peres and Oliva-Teles 1999a). These workers observed that fish fed diets containing 12–24% lipid appeared to regulate protein intake rather than energy intake. Results of other studies using isocaloric diets also demonstrated that, under laboratory conditions, dry matter feed intake of channel catfish (Lim and Klesius 1998a, 1998b) and European sea bass (Peres and Oliva-Teles 1999b) significantly decreased with increasing level of dietary protein to a certain limit. Under pond conditions, Li and Lovell (1992a, 1992b) showed that feed consumption of channel catfish fed to apparent satiation with isocaloric diets decreased linearly as dietary protein concentration increased.

Increases in tissue lipid deposition and reduction in moisture content have been reported in fish fed increasing dietary lipid levels (Watanabe 1982; Williams and Robinson 1988; Tidwell and Robinette 1990; Gatlin and Bai 1993; NRC 1993; Wilson and Moreau 1996; Twibell and Wilson 2003; Lim et al. 2006). Gatlin and Stickney (1982), however, obtained similar carcass lipid percentages of young channel catfish fed diets containing the same lipid source regardless of the percentage (6–14%) of lipid in the diet. In the present study, whole-body and fillet lipid levels increased, whereas moisture contents decreased in catfish fed diets containing increasing levels of supplemental fish oil. Increases in tissue lipid concentrations were likely a result of higher dietary energy content because of the addition of fish oil. Lipid concentrations in commercial channel catfish diets are generally limited to 5–6% as higher levels can result in increased fat deposition in edible tissues and the visceral cavity (Wilson and Moreau 1996). NRC (1993) reported that excess dietary energy led to deposition of large amounts of fat in tissues. Increasing feeding duration from 3 to 15 wk also resulted in increased lipid deposition and decreased moisture content in fillets. This may be related to fish age or size. Froyland et al. (2000) reported that juvenile fish possess a higher FA catabolism, and this might explain why younger fish have relatively less body lipid compared with older fish.

Percentage of whole-body and fillet protein decreased as supplemental fish oil levels increased to 6% or higher. This may be because of higher levels of tissue lipid in fish fed diets with increasing levels of supplemental fish oil. Lower protein concentration of whole fish fed high-lipid diets has also been reported for rainbow trout (Chaiyapechara et al. 2003). Page and Andrews (1973) suggested that lower whole-body protein in channel catfish fed high-fat diets was a result of dilution with lipid. The significantly lower fillet protein content of catfish sampled at Week 3 than those sampled at Week 6 or thereafter could be related to fish size. Shearer (1994) indicated that protein is a stable component of fish body with respect to diets and feeding levels but is dependent mainly on fish weight. It usually increases with fish size, remaining stable after a certain size is reached. For Atlantic salmon, carcass protein level stabilizes when fish reach 100 g in weight (Shearer et al. 1994).

FA compositions of whole body and fillets were influenced by dietary FA composition as has been demonstrated in earlier studies for various fish species (Stickney and Andrews 1971; Williams and Robinson 1988; Morais et al. 2001; Peng et al. 2003; Schulz et al. 2005; Yildiz et al. 2005). Marked changes in tissue FA composition as a response to increasing dietary fish oil levels were increased levels (either as percentage of total FA or mg/g of fillet) of n-3 FA, particularly n-3 HUFA (EPA, 20:5 n-3; DHA, 22:6 n-3) rather than linolenic acid (18:3 n-3). Increased levels of DHA and EPA in fillets of catfish fed a practical diet supplemented with 0, 1.5, and 3% menhaden fish oil were also reported by Morris et al. (1995). Concentrations of linolenic acid in all analyzed tissues ranged from 0.68 to 1.21% and remained relatively constant throughout the 15-wk feeding period. These values, however, were similar to that obtained by Tidwell and Robinette (1990) (0.77%) but higher than that reported by Robinson et al. (2001) (0.28%) for pond-raised channel catfish fillets. Among n-3 HUFA, the proportion of DHA retained (relative to the levels present in diets) in channel catfish tissues (whole body and fillets) was higher than that of EPA. Torstensen et al. (2004) reported that in Atlantic salmon, DHA was more effectively retained in liver than in muscle. It has been suggested that salmonid metabolism can discriminate between dietary FAs, employing them for selective deposition or energy production (Roselund et al. 2001; Bell et al. 2002). This indicates a selective beta-oxidation of EPA over DHA (Madsen et al. 1998) because of the complex catabolism of DHA (Bell et al. 2001) and/or possibly production of DHA from EPA by desaturase and elongase activity (Tocher et al. 1997). This phenomenon has not been studied in channel catfish. However, the selective retention of DHA over EPA may have also occurred in channel catfish because higher proportions of dietary DHA were retained in tissues.

Other noticeable changes as a result of increasing dietary fish oil levels were decreasing concentrations of monoenes and total n-6 and increasing total saturated FA. These values for total monoenes, n-6, and saturated FA were similar, lower, and higher, respectively, than those of fillets from pond-raised channel catfish reported by Robinson et al. (2001). Among monoenoic FAs, oleic acid (18:1 n-9) was the primary FA in all analyzed tissue lipids and accumulated at levels higher than those found in dietary lipids. Linoleic acid (18:2 n-6) was the principal n-6 FA in all analyzed tissues but deposited at levels lower than those in diets. A portion of dietary 18:2 n-6 may be desaturated and/or chain elongated to 20:2 n-6, 18:3 n-6, 20:3 n-6, 20:4 n-6, and 22:5 n-6 because tissue contents of these FA were generally higher than those found in diets. It is known that some freshwater fish are able to desaturate and elongate dietary linoleic acid to produce longer chain FAs (Olsen et al. 1990; Tocher et al. 2002; Maina et al. 2003).

Fillet percentages of saturates significantly decreased, whereas those of monoenes increased with increasing feeding periods up to 9 and 12 wk, respectively. Total n-6 FA was also significantly affected by feeding period and the interaction between dietary fish oil levels and feeding period. These effects were attributed to the increased percentage of n-6 FA in fillets of fish sampled at Week 9. This was unexpected and could not be explained because n-6 fillet contents sampled prior to and after Week 9 (Weeks 3, 6, 12, and 15) were similar.

Total n-3 FA contents and n-3/n-6 ratios remained statistically similar throughout various feeding periods. Percentages of n-3 HUFA, however, significantly decreased at Weeks 9, 12, and 15 and ranged from 8.2 to 9.0%. These values, however, were considerably higher than 2.8% for fillets of pond-grown channel catfish reported by Robinson et al. (2001). This reduction of n-3 HUFA was attributed to decreasing levels of this group of FA in fillets of fish fed the control and 3% added fish oil diets as feeding duration increased. Fish used in our study were grown under laboratory conditions from yolk sac fry to juveniles on commercial trout fry diets, which are known to contain high levels of lipid rich in n-3 HUFA. Even though they were fed the experimental control diet for 2 wk prior to the beginning of the study, n-3 HUFA content of the initial fish remained relatively high (10.06%). As the feeding experiment progressed, fillet n-3 HUFA contents of fish fed the control and 3% added fish oil diets continued to gradually decrease and reached the lowest concentrations at Weeks 9 and 12 for fish fed the 3% fish oil and control diets, respectively. The group fed 6 or 9% supplemental fish oil diets had higher or comparable levels of n-3 HUFA to that of the initial fish. This suggests that n-3 HUFA present in the control and 3% fish oil diets were insufficient to maintain the initial concentration of these FA. A supplemental fish oil level of 6% was sufficient to elevate or at least maintain constant levels of fillet n-3 HUFA. The ratio of n-3/n-6, although not significantly affected by feeding duration, numerically decreased after Week 6. This suggests that feeding channel catfish the diet supplemented with 6% fish oil longer than 6 wk might not be beneficial in terms of increasing fillet content of n-3 HUFA. When expressed in mg/g of fillet, the highest level of total n-3 FA was obtained in fish fed the highest (9%) added fish oil diet for the longest (15 wk) time period.

Incorporation of FAs into fish tissues, however, is modulated by various metabolic factors, and final composition will depend upon the initial FA content, cumulative intake of dietary FAs, growth rate, and duration of feeding. With fast-growing young fish, it is possible to obtain a desirable effect of dietary FAs on fish FA composition in a relatively short period of time. In large fish, because the relative weight increment is small, the initial FA composition will continue to have a strong influence on final composition. Bell et al. (2003) reported that returning to a diet containing solely fish oil after feeding diets with various levels of rapeseed oil to Atlantic salmon restored the values of DHA and EPA to levels of those of fish fed only the fish oil diet for 12 wk. However, linoleic acid and the ratio of n-3/n-6 FA were not restored.

Results of this study indicate that supplementation of menhaden fish oil at levels up to 9% to a commercial catfish diet had no effect on growth, feed intake, utilization efficiency, and survival of juvenile channel catfish reared under laboratory conditions. Increasing supplemental fish oil levels increased tissue lipid and decreased moisture and protein contents. Tissue FA contents, particularly n-3 and n-3 HUFA, and the ratio of n-3/n-6 FA increased with increasing dietary fish oil levels. Feeding juvenile channel catfish the 6% added fish oil diet for 6 wk appeared to be sufficient to maintain desirable n-3/n-6 FA ratios and n-3 HUFA levels, which are beneficial to human health. However, maximum n-3 FA (in mg/g of fillet) was obtained in fish fed the highest (9%) fish oil diet for 15 wk. Although increasing levels of n-3 FA in catfish fillets can be accomplished by feeding diets supplemented with menhaden fish oil at various time periods, feeding high levels of fish oil diets has been reported to impart objectionable fishy flavor (Morris et al. 1995). Thus, it is suggested that the maximum amount of n-3 FA in fillets that do not adversely affect flavor and is acceptable by consumers be established as a means of producing catfish that may reduce the risk of cardiovascular disease.

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